Suitability of Lychee Fruits On and Off the Tree for *Cryptophlebia* spp.

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Abstract. *Cryptophlebia illepida* and *C. ombrodelta* larval establishment was studied in lychee fruit on and off the tree under field conditions. The proportion of larvae establishing from hatched eggs and the proportion of fruit with at least one larva establishing were significantly higher for *C. illepida* in fruit detached from the tree compared with fruit on the tree. The trend for *C. ombrodelta* was toward higher establishment in fruit on the tree compared with fruit detached from the tree but results were not significant. Although lychee is considered a poor host for *C. illepida*, the threat of introducing this pest in contraband fruit entering the U.S. mainland may be greater than previously thought.

Key words: koa seedworm, litchi fruit moth, host suitability, quarantine pest

Introduction

The lychee fruit moth, *Cryptophlebia ombrodelta* (Lower) and the koa seedworm, *C. illepida* (Butler) (Lepidoptera: Tortricidae), are quarantine pests that limit exports of lychee and longan from Hawaii to the U.S. mainland. Estimates of pest risk and likelihood of introduction for a quarantine pest are typically based on field infestation studies due to a lack of detailed postharvest information. These studies assume that the infestation rate at some future point when the fruit arrives at an export destination is equal to the infestation rate at harvest, which ignores pest survivorship on the host after harvest. For certain quarantine pests, survivorship on the host is important in calculating pest risk potential and the level of acceptable quarantine security when developing a postharvest quarantine treatment (Follett and McQuate 2001).

Lychee is considered to be a poor host for *Cryptophlebia*. Eggs are laid on fruit of all stages of maturity. In mature fruit, newly emerged larvae bore through the skin and feed at the skin-pulp interface before attempting to bore to the seed. If the larva bores toward the seed at the peduncle, it can reach the seed and survive, but if the larva attempts to bore through the mature flesh it usually drowns in the juice that seeps in from the wound caused by its feeding (Waite and Hwang 2002). If larvae reach the seed, most of the seed may be consumed as they mature before leaving the fruit to pupate. In a survey on the island of Hawaii that sampled 35,722 harvest-mature lychee fruits of six cultivars, 396 *Cryptophlebia* larvae were reared from 383 infested fruits for an infestation rate of 1.1% (G. McQuate, unpublished data). Both *Cryptophlebia* species were identified from fruit but the species ratio was not determined. In a two-year survey that sampled 3,197 mature lychee fruits on Kauai and the island of Hawaii in 1998, 36% of fruit had eggs, 3% had young larvae and 5% showed feeding damage, but late instars were rarely observed (Suverkropp and Jones, unpublished data). On Kauai in 1997, an average of 15% of *Cryptophlebia* caught in traps during the study were *C. illepida*, whereas in 1998 55% were *C. illepida*. In 1998 on the Big Island, trapping of *C. illepida* versus *C. ombrodelta* varied among orchards between 19-92% (Suverkropp and Jones, unpublished data).

A preliminary study where young larvae were placed on individual detached fruit in the laboratory resulted in better survival to pupation than was observed in the field, suggesting harvested fruit might be a more suitable host. Here we report on a study conducted to examine the difference between *C. illepida* and *C. ombrodelta* larval survival in fruit on and off the tree under comparable conditions. Our hypothesis was that lychee fruit detached from the tree (dropped or harvested fruit) are more suitable for *Cryptophlebia* larval development than fruit attached to the tree.

Methods

Natural infestation by *Cryptophlebia*. All field experiments were conducted on 'Kaimana' lychee at a commercial orchard in the Panaewa area of Hilo, HI. A survey was conducted of the orchard on 9 June 2001 to determine natural levels of *Cryptophlebia* infestation. Ten large green (not ripe) fruits from 20 trees (n = 200) were randomly collected and brought back to the laboratory for inspection. The fruit weighed 2.65 kg for an average fruit weight of 13.3 g. Under a dissecting microscope, the outer surface of each fruit was examined for *Cryptophlebia* eggs and external frass (evidence of *Cryptophlebia* larval feeding within). For those fruit where eggs or frass were found, the number of eggs per fruit was recorded and the fruit was placed inside a 2 l plastic container lined with sand to rear out *Cryptophlebia*.The fruit were checked for larval establishment at 2 weeks (23 June 2001). Whenever frass was found.the fruit was dissected. Dissection started at the entry hole and the entire fruit was examined for larvae. Larvae from lychee were reared to adults on artificial diet in individual cups to determine the species of *Cryptophlebia*. In those fruit where no eggs or frass were found, fruit were transferred to a plastic container; and held until any *Cryptophlebia* that were overlooked could emerge, and to observe for other insects, such as fruit flies.

Larval survivorship in fruit on and off the tree. Approximately 20 trees with fruit of suitable maturity were identified. Panicles with 6–8 _-ripe fruits were caged and randomly assigned to treatments. All field cages were set-up over panicles with similar numbers of fruit. The body of the cage was lightweight plastic (10 cm height, 20 cm diameter) with organza tops and bottoms that could be opened easily and cinched shut. Cage temperatures were 0.5–1.0°C above ambient temperatures for the duration of the experiment. The factorial design included *C. illepida* and *C. ombrodelta*, two treatments (on and off the tree), and two harvest times (2 and 4 weeks). The experiment was repeated two times separated by 1 week, which required the entire duration of the lychee fruiting season. Five gravid females from laboratory colonies (Follett and Lower 2000) were placed in each cage for 24 h to initiate the infestation. Half of the field cages received *C. illepida* and half *C. ombrodelta* moths. At the time moths were removed, fruit on panicles were simply left as is for the "on-tree" (attached) treatment; for the fruit in the "off-tree" (detached) treatment, fruit stems were cut from the panicle then re-suspended from the panicle using paper clips. Therefore, the only difference between treatments after oviposition was the form of attachment of fruit (stems or paper clips) to the panicle. Fruit were removed from cages at 2 or 4 weeks and dissected. Each treatment was replicated 4 times on each date (32 cages on each date). Data were taken on the number of eggs per fruit, egg hatch, and the number of larvae inside fruit and their life stage.

Statistical analysis. We analyzed only the data for the fruits that were removed from the cages after 2 weeks. The data from the fruits removed from the cages after 4 weeks were not analyzed because many fruit in the attached treatment had ripened and abscised naturally. An unusually high number of eggs were laid on a few fruits in the cages. Moths rarely lay >10 eggs on a single fruit during natural oviposition (PAF, personal observation), so we also excluded from the analysis any fruit that carried more than 10 eggs.

Data on the establishment of larvae on fruit were analyzed using a nominal response model (SAS Institute, 2002). We classified the data into two categories by separating those fruits with 1 or more larvae and those fruits with no larvae into two groups. We analyzed the full model for three independent factors: (1) The two species *C. illepida* and *C. ombrodelta*, (2) treatment of having the fruit attached to or detached from the panicle, and (3) a blocking factor for the time that the experiment was initiated. Data on the proportion of neonates establishing was subjected to analysis of variance (ANOVA) using the standard least squares model to test for differences in treatment effects. When treatment effects were significant, means separations were done using a Wilcoxon rank-sum test at $P \leq 0.05\%$. To examine the relationship between the number of eggs laid on the fruit and the probability of larvae establishing on the fruit, we performed a contingency analysis using maximum likelihood with the number of hatched eggs on a fruit as the independent variable and the presence-absence of established larvae as the dependent variable. We performed contingency analysis for each species and treatment separately combining data from experimental runs.

Results

Natural *Cryptophlebia* infestation. Forty-nine out of 200 fruit had eggs and/or larvae for an infestation rate of 24.5%. A total of 62 eggs were found, or 1.27 eggs per fruit, and the greatest number of eggs per fruit was three. After two weeks, 16.3% of the infested fruit had live larvae inside. All larvae from infested fruit were identified as *C. ombrodelta* when reared to adult. No *C. illepida*, fruit flies or other pests were observed.

Larval survivorship in fruit on and off the tree. Significant differences in the rate of fruit infestation and the rate of neonate establishment were observed for the effects of time (date of experiment) and species by treatment (fruit attached to or detached from the panicle) (Table 1). Means separations were done on the two experiment dates separately for each species. In the first experiment, the percent of fruit with >1 larvae establishing and the percent of hatched eggs (i.e., neonates) successfully establishing was not significantly different for either species in the attached fruit or detached fruit treatments (Table 2). However, the trend was for higher *C. illepida* establishment in fruit detached from the panicle, and higher *C. ombrodelta* establishment in fruit attached the panicle. The trend was more pronounced when the experiment was repeated. In the second the experiment, *C. illepida* established in significantly more fruit ($c^2 = 7.8$, P = 0.005), and the rate of establishment of neonates was significantly higher in fruit detached from the panicle compared with fruit attached to the panicle ($Z = 3.4$, P = 0.0006) (Table 2). Conversely, *C. ombrodelta* established in significantly more fruit ($c^2 = 8.1$, P = 0.005) when fruit were attached to the panicle compared with fruit that were detached from the panicle. The number of eggs per fruit was significantly less for *C. ombrodelta* in detached fruit treatment compared with the attached fruit treatment. Contingency table analysis showed an increase in the rate of establishment for *C. illepida* when more than one egg hatched on the fruit compared with when only one egg hatched in the attached fruit treatment ($c^2 = 31.6$, P = 0.0005) but not the detached fruit treatment. Conversely, the rate of establishment for *C. ombrodelta* increased when more than one egg hatched compared with when only one egg hatched in the detached fruit treatment ($c^2 = 9.7$, P = 0.04), but not the attached fruit treatment.

Discussion

Our hypothesis that lychee fruit off the tree (dropped or harvested fruit) are more suitable for larval development than fruit on the tree appears to be true for *C. illep*ida. In the first and second experiments, larval establishment was higher for *C. illepida* in fruit detached from

Table 1. Statistical analyses of the proportion of larvae establishing on fruit from hatched eggs and the proportion of fruit with at least one larva establishing using the full factorial model.

		Proportion of larvae establishing on fruit from hatched eggs [1]			Proportion of fruit with at least one larva establishing [2]	
Source	**df**	**SS**	**F ratio**	**Prob> F**	**Wald χ^2**	**Prob> χ^2**
Species	1	0.0063	0.048	0.827	0.796	0.372
Time (block)	1	1.1420	8.552	0.004**	9.136	0.003**
Species*time	1	0.0716	0.536	0.465	0.150	0.699
Treatment (on/off)	1	0.4476	3.352	0.069	0.007	0.935
Species* treatment	1	1.1074	8.292	0.005**	8.035	0.005**
Time* treatment	1	0.0269	0.201	0.654	0.138	0.711
Species*time* treatment	1	0.1259	0.943	0.333	1.625	0.202

[1]ANOVA; ** significant at P<0.01
[2]Ordinal logistic model using maximum likelihood; ** significant at P<0.01

Table 2. Establishment of *Cryptophlebia* spp. in lychee fruit on and off the tree.

Species	Date	Trmt.	Eggs per fruit	Hatched eggs per fruit	Establishment[1] of larvae on fruit	
					% Fruit with larvae	% Neonates establishing
C. illepida	1	off	3.6a	3.6	41.2a	30.7a
		on	4.9a	4.3	31.3a	11.6a
	2	off	4.5a	4.2	75.0a	51.7a
		on	4.6a	4.6	38.9b	16.1b
C. ombrodelta	1	off	1.5a	1.4	21.1a	16.7a
		on	4.3b	3.6	33.3a	19.7a
	2	off	2.2a	1.7	38.5a	34.8a
		on	3.6b	3.4	68.4b	44.0a

[1]Establishment means that the neonate developed to second instar or further.
Column means followed by the same letter for each species on each experiment date are not significantly different (P = 0.05) by a Wilcoxon rank-sum test (eggs per fruit and % neonates establishing), or c^2 test (% fruit with larvae).

the tree compared with fruit attached to the tree, and this effect was highly significant in the second experiment. The reason for higher survivorship in detached fruit is unknown but possibly when fruit are detached from the panicle the fruit begins to dry and fewer larvae drown when they bore into the fruit and feed on the pulp.

Results with *C. ombrodelta* are less clear. For *C. ombrodelta*, percent of fruit with established larvae and percent neonates establishing are numerically higher in the attached fruit treatment compared with the detached fruit treatment for both experiments, and the treatment effect on percent fruit with larvae was significant in the second experiment. However, the number of eggs occurring on fruit in the detached fruit treatment was significantly lower than in the attached fruit treatment in both experiments, which may have influenced the results. Contingency analysis showed that larval establishment on a fruit was more likely if more than 1 egg was laid and hatched, although the relationship was inconsistent among treatments. For *C. ombrodelta* in the detached fruit treatment, many fruit had 1 (58% of fruit) or 2 (26% of fruit) eggs (average 1.5-2.2 eggs/fruit), whereas 74% of the fruit in the attached fruit treatment had more than 1 egg (average 3.6-4.3 eggs/fruit). Therefore, the greater number of eggs on fruit in the attached fruit treatment may have enhanced larval establishment of *C. ombrodelta*, and caution must be taken in drawing conclusions about treatment effects. Equal numbers of eggs occurred on fruit in the *C. illepida* treatments (attached and detached) so this was not a confounding factor.

Commercial shipments of lychee to the U.S. mainland receive a postharvest quarantine treatment before export to disinfest fruit of fruit flies and *Cryptophlebia*. However, contraband fruit that may be carried in passenger baggage or sent by mail is a persistent problem, and untreated lychee fruits are sometimes intercepted in U.S. ports. Results show that *C.*

illepida survivorship improves 3-fold in harvested lychee fruit compared to survivorship in fruit on the tree. Therefore, although lychee is considered a poor host for *C. illepida* the threat of introducing this pest in contraband fruit entering the U.S. mainland may be greater than previously thought.

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